

| L Number | Hits | Search Text | DB | Time stamp |
|----------|------|---|--------------------|------------------|
| (LI) | 80 | (lectin or hemaggluinat\$ or ricin) near3 resist\$8 | USPAT; US-PGPUB | 2003/09/15 15:31 |

PGPUB-DOCUMENT-NUMBER: 20030115614

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030115614 A1

TITLE: Antibody composition-producing cell

PUBLICATION-DATE: June 19, 2003

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|--------------------|-------|-------|---------|---------|
| Kanda, Yutaka | Tokyo | | JP | |
| Satoh, Mitsuo | Tokyo | | JP | |
| Nakamura, Kazuyasu | Tokyo | | JP | |
| Uchida, Kazuhisa | Tokyo | | JP | |
| Shinkawa, Toyohide | Tokyo | | JP | |
| Yamane, Naoko | Tokyo | | JP | |
| Hosaka, Emi | Tokyo | | JP | |
| Yamano, Kazuya | Tokyo | | JP | |
| Yamasaki, Motoo | Tokyo | | JP | |
| Hanai, Nobuo | Tokyo | | JP | |

APPL-NO: 09/ 971773

DATE FILED: October 9, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60268916 20010216 US

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | DOC-ID | APPL-DATE |
|---------|----------------|-----------------------|-----------------|
| JP | 2000-308526 | 2000JP-2000-308526 | October 6, 2000 |
| WO | PCT/JP01/08804 | 2001WO-PCT/JP01/08804 | October 5, 2001 |

US-CL-CURRENT: 800/6, 435/326 , 435/358

ABSTRACT:

The present invention relates to a cell for the production of an antibody molecule such as an antibody useful for various diseases having high antibody-dependent cell-mediated cytotoxic activity, a fragment of the antibody and a fusion protein having the Fc region of the antibody or the like, a method for producing an antibody composition using the cell, the antibody composition and use thereof.

----- KWIC -----

Summary of Invention Paragraph - BSTX (19):

[0018] Mutants regarding the activity of an enzyme relating to the modification of a sugar chain are mainly selected and obtained as a **lectin-resistant** cell line. For example, CHO cell mutants having various sugar chain structures have been obtained as a **lectin-resistant** cell line using a lectin such as WGA (wheat-germ agglutinin derived from *T. vulgaris*), ConA (concanavalin A derived from *C. ensiformis*), RIC (a toxin derived from *R. communis*), L-PHA (leucoagglutinin derived from *P. vulgaris*), LCA (lentil agglutinin derived from *L. culinaris*), PSA (pea lectin derived from *P. sativum*) or the like [Somatic Cell Mol. Genet., 12, 51 (1986)].

Summary of Invention Paragraph - BSTX (68):

[0066] (e) a technique for selecting a cell line **resistant to a lectin** which recognizes a sugar chain in which 1-position of fucose is bound to 6-position of N-acetylglucosamine in the reducing end through .alpha.-bond in the complex N-glycoside-linked sugar chain.

Summary of Invention Paragraph - BSTX (69):

[0067] (16) The CHO cell according to any one of (4) to (15), which is **resistant to at least a lectin** which recognizes a sugar chain in which 1-position of fucose is bound to 6-position of N-acetylglucosamine in the reducing end through .alpha.-bond in the complex N-glycoside-linked sugar chain.

Summary of Invention Paragraph - BSTX (120):

[0118] (35) The cell according to any one of (23) to (34), which is **resistant to at least a lectin** which recognizes a sugar chain in which 1-position of fucose is bound to 6-position of N-acetylglucosamine in the reducing end through .alpha.-bond in the N-glycoside-linked sugar chain.

Brief Description of Drawings Paragraph - DRTX (44):

[0227] FIG. 43 shows ADCC activities of anti-CCR4 human chimeric antibodies produced by **lectin-resistant** cell lines. The ordinate and the abscissa show the cytotoxic activity and the antibody concentration. ".quadrature.", ".box-solid.", ".diamond-solid." and ".tangle-solidup." show the activities of antibodies produced by the strain 5-03, CHO/CCR4-LCA, CHO/CCR4-AAL and CHO/CCR4-PHA, respectively.

Brief Description of Drawings Paragraph - DRTX (45):

[0228] FIG. 44 shows ADCC activities of anti-CCR4 human chimeric antibodies produced by **lectin-resistant** cell lines. The ordinate and the abscissa show the cytotoxic activity and the antibody concentration, respectively. ".quadrature.", ".DELTA." and ".circle-solid." show activities of antibodies produced by YB2/0 (KM2760 # 58-35-16), 5-03 and CHO/CCR4-LCA, respectively.

Detail Description Paragraph - DETX (83):

[0321] (e) a technique for selecting a cell line resistant to a lectin which recognizes a sugar chain in which 1-position of fucose is bound to 6-position of N-acetylglucosamine in the reducing end through .alpha.-bond in the complex N-glycoside-linked sugar chain, and the like.

Detail Description Paragraph - DETX (84):

[0322] Herein, the lectin-resistant cell line can be obtained by culturing a cell line in a medium comprising a predetermined concentration of lectin and then by selecting a cell line which acquires such a property that its survival rate is increased at least 2 times, preferably 3 times, and more preferably 5 times or more, than the parent cell line with statistical significance. Also, it can also be obtained by culturing a cell line in a medium comprising lectin and then by selecting a cell line which can be cultured at a certain survival rate, e.g., 80% survival rate, at a lectin concentration of at least 2 times, preferably 5 times, more preferably 10 times, and most preferably 20 times or more, than the parent cell line.

Detail Description Paragraph - DETX (221):

[0459] (5) Method for Selecting a Cell Line Resistant to a Lectin which Recognizes a Sugar Chain Structure in which 1-Position of Fucose is Bound to 6-Position of N-Acetylglucosamine in the Reducing End Through .alpha.-Bond in the N-Glycoside-Linked Sugar Chain

Detail Description Paragraph - DETX (222):

[0460] The host cell of the present invention can be prepared by using a method for selecting a cell line resistant to a lectin which recognizes a sugar chain structure in which 1-position of fucose is bound to 6-position of N-acetylglucosamine in the reducing end through .alpha.-bond in the N-glycoside-linked sugar chain.

Detail Description Paragraph - DETX (223):

[0461] Examples of the method for selecting a cell line resistant to a lectin which recognizes a sugar chain structure in which 1-position of fucose is bound to 6-position of N-acetylglucosamine in the reducing end through .alpha.-bond in the N-glycoside-linked sugar chain include the methods using lectin described in Somatic Cell Mol. Genet., 12, 51 (1986) and the like. As the lectin, any lectin can be used, so long as it is a lectin which recognizes a sugar chain structure in which 1-position of fucose is bound to 6-position of N-acetylglucosamine in the reducing end through .alpha.-bond in the N-glycoside-linked sugar chain. Examples include a Lens culinaris lectin LCA (lentil agglutinin derived from Lens culinaris), a pea lectin PSA (pea lectin derived from Pisum sativum), a broad bean lectin VFA (agglutinin derived from Vicia faba), an Aleuria aurantia lectin AAL (lectin derived from Aleuria aurantia) and the like.

Detail Description Paragraph - DETX (224):

[0462] Specifically, the cell line of the present invention **resistant to a lectin** which recognizes a sugar chain structure in which 1-position of fucose is bound to 6-position of N-acetylglucosamine in the reducing end through .alpha.-bond in the N-glycoside-linked sugar chain can be selected by culturing cells for 1 day to 2 weeks, preferably from 1 day to 1 week, using a medium comprising the lectin at a concentration of 1 .mu.g/ml to 1 mg/ml, subculturing surviving cells or picking up a colony and transferring it into a culture vessel, and subsequently continuing the culturing using the lectin-containing medium. Examples of the cell line obtained by the method include CHO/CCR4-LCA Nega-13 (FERM BP-7756) obtained in Example 14(2) which will be described later.

Detail Description Paragraph - DETX (767):

[0991] Preparation of **Lectin-Resistant** CHO/DG44 Cell and Production of Antibody Using the Cell:

Detail Description Paragraph - DETX (768):

[0992] (1) Preparation of **Lectin-Resistant** CHO/DG44

Detail Description Paragraph - DETX (769):

[0993] CHO/DG44 cells were grown until they reached a stage of just before confluent, by culturing in a 75 cm.sup.2 flask for adhesion culture (manufactured by Greiner) using IMDM-FBS(10) medium [IMDM medium comprising 10% of fetal bovine serum (FBS) and 1.times. concentration of HT supplement (manufactured by GIBCO BRL)]. After washing the cells with 5 ml of Dulbecco PBS (manufactured by Invitrogen), 1.5 ml of 0.05% trypsin (manufactured by Invitrogen) diluted with Dulbecco PBS was added thereto and incubated at 37.degree. C. for 5 minutes for peel the cells from the flask bottom. The peeled cells were recovered by a centrifugation operation generally used in cell culture and suspended in IMDM-FBS(10) medium to give a density of 1.times.10.sup.5 cells/ml, and then 0.1 .mu.g/ml of an alkylating agent N-methyl-N'-nitro-N-nitrosoguanidine (hereinafter referred to as "MNNG", manufactured by Sigma) was added or not added thereto. After incubating them at 37.degree. C. for 3 days in a CO.sub.2 incubator (manufactured by TABAI), the culture supernatant was discarded, and the cells were again washed, peeled and recovered by the same operations, suspended in IMDM-FBS(10) medium and then inoculated into an adhesion culture 96 well plate (manufactured by IWAKI Glass) to give a density of 1,000 cells/well. To each well, as the final concentration in medium, 1 mg/ml Lens culinaris agglutinin (hereinafter referred to as "LCA", manufactured by Vector), 1 mg/ml Aleuria aurantia agglutinin (Aleuria aurantia lectin; hereinafter referred to as "AAL", manufactured by Vector) or 1 mg/ml kidney bean agglutinin (Phaseolus vulgaris leucoagglutinin; hereinafter referred to as "L-PHA", manufactured by Vector) was added. After culturing them at 37.degree. C. for 2 weeks in a CO.sub.2 incubator, the appeared colonies were obtained as **lectin-resistant** CHO/DG44. Regarding the obtained **lectin-resistant** CHO/DG44, an LCA-resistant cell line was named CHO-LCA, an AAL-resistant cell line was named CHO-AAL and an L-PHA-resistant cell line was named CHO-PHA. When the resistance of these cell lines to various kinds of lectin was examined, it was found that the CHO-LCA was also resistant to AAL and the CHO-AAL was also resistant LCA. In addition,

the CHO-LCA and CHO-AAL also showed a **resistance to a lectin** which recognizes a sugar chain structure identical to the sugar chain structure recognized by LCA and AAL, namely a lectin which recognizes a sugar chain structure in which 1-position of fucose is bound to 6-position of N-acetylglucosamine residue in the reducing end through .alpha.-bond in the N-glycoside-linked sugar chain. Specifically, it was found that the CHO-LCA and CHO-AAL can show resistance and survive even in a medium supplemented with 1 mg/ml at a final concentration of a pea agglutinin (Pisum sativum agglutinin; hereinafter referred to as "PSA", manufactured by Vector). In addition, even when the alkylating agent MNNG was not added, it was able to obtain **lectin-resistant** cell lines by increasing the number of cells to be treated. Hereinafter, these cell lines were used in analyses.

Detail Description Paragraph - DETX (771):

[0995] An anti-CCR4 human chimeric antibody expression plasmid pKANTEX2160 was introduced into the three **lectin-resistant** cell lines obtained in the (1) by the method described in Example 8, and gene amplification by a drug MTX was carried out to prepare an anti-CCR4 human chimeric antibody-producing cell line. By measuring an amount of antibody expression by the ELISA described in Example 8-2, antibody-expressing transformants were obtained from each of the CHO-LCA, CHO-AAL and CHO-PHA. Regarding each of the obtained transformants, a transformant derived from CHO-LCA was named CHO/CCR4-LCA, a transformant derived from CHO-AAL was named CHO/CCR4-AAL and a transformant derived from CHO-PHA was named CHO/CCR4-PHA. Also, the CHO/CCR4-LCA, as a name of Nega-13, has been deposited on Sep. 26, 2001, as FERM BP-7756 in International Patent Organism Depositary, National Institute of Advanced Industrial Science and Technology (AIST Tsukuba Central 6, 1-1, Higashi 1-Chome Tsukuba-shi, Ibaraki-ken 305-8566 Japan)).

Detail Description Paragraph - DETX (772):

[0996] (3) Production of Potent ADCC Activity Antibody by **Lectin-Resistant** CHO Cell

Detail Description Paragraph - DETX (774):

[0998] (4) Sugar Chain Analysis of Antibodies Produced by **Lectin-Resistant** CHO Cell

Detail Description Paragraph - DETX (790):

[1012] Results of the sugar chain structure analysis of the purified anti-CCR4 human chimeric antibodies produced by **lectin-resistant** cell lines are shown in Table 6. The result shows the analysis of sugar chains of the anti-CCR4 human chimeric antibody produced by **lectin-resistant** cell lines. The ratio of .alpha.-1,6-fucose-free sugar chains (%) calculated from peak areas by analyzing by the method described in Example d(4) is shown in the table.

Detail Description Paragraph - DETX (793):

[1014] Analysis of **Lectin-Resistant** CHO Cell Line:

Detail Description Paragraph - DETX (828):

[1049] (4) Measurement of Lectin Resistance in Transformed Cells

Claims Text - CLTX (16):

15. The CHO cell according to any one of claims 4 to 14, wherein the enzyme activity is decreased or deleted by a technique selected from the group consisting of the following (a), (b), (c), (d) and (e): (a) a gene disruption technique targeting a gene encoding the enzyme; (b) a technique for introducing a dominant negative mutant of a gene encoding the enzyme; (c) a technique for introducing mutation into the enzyme; (d) a technique for inhibiting transcription and/or translation of a gene encoding the enzyme; (e) a technique for selecting a cell line resistant to a lectin which recognizes a sugar chain in which 1-position of fucose is bound to 6-position of N-acetylglucosamine in the reducing end through .alpha.-bond in the complex N-glycoside-linked sugar chain.

Claims Text - CLTX (17):

16. The CHO cell according to any one of claims 4 to 15, which is resistant to at least a lectin which recognizes a sugar chain in which 1-position of fucose is bound to 6-position of N-acetylglucosamine in the reducing end through .alpha.-bond in the complex N-glycoside-linked sugar chain.

Claims Text - CLTX (36):

35. The cell according to any one of claims 23 to 34, which is resistant to at least a lectin which recognizes a sugar chain in which 1-position of fucose is bound to 6-position of N-acetylglucosamine in the reducing end through .alpha.-bond in the N-glycoside-linked sugar chain.

PGPUB-DOCUMENT-NUMBER: 20020197705

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020197705 A1

TITLE: Methods to identify mutant cells with altered sialic acid

PUBLICATION-DATE: December 26, 2002

INVENTOR-INFORMATION:

| NAME | CITY | STATE | COUNTRY | RULE-47 |
|--------------------|---------|-------|---------|---------|
| Kawaoka, Yoshihiro | Madison | WI | US | |

APPL-NO: 10/ 081170

DATE FILED: February 22, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60271044 20010223 US

US-CL-CURRENT: 435/235.1, 435/325 , 435/349 , 435/350

ABSTRACT:

The invention provides cells useful to propagate influenza virus mutants having reduced sialidase activity.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of the filing date of U.S. application Serial No. 60/271,044, filed on Feb. 23, 2001, under 35 U.S.C. .sctn.119(e), the disclosure of which is incorporated by reference herein.

----- KWIC -----

Pre-Grant Publication Document Identifier - DID (1):

US 20020197705 A1

Summary of Invention Paragraph - BSTX (10):

[0010] As described hereinbelow, cell lines were generated that expressed reduced levels of the influenza viral receptor determinant, sialic acid, by selecting Madin-Darby canine kidney cells which were resistant to a lectin specific for sialic acid linked to galactose by .alpha.(2-3) or .alpha.(2-6) linkages. One of these cell lines, MaKS, had less than [fraction (1/10)] as much N-acetylneuraminic acid as its parental cell line, MDCK. When serially

passed in MaKS, human H3N2 viruses lost sialidase activity due to a large internal deletion in the NA gene, without alteration of the HA gene. These findings indicate that NA mutations can contribute to the adaptation of influenza A virus to new host environments and hence may play a role in the transmission of virus across species.

Brief Description of Drawings Paragraph - DRTX (2):

[0015] FIG. 1. Binding of **lectin-resistant** cell lines. For each cell line, cells were incubated with digoxigenin-labeled Maackia amurensis (MAA) or Sambucus nigra (SNA) lectins, followed by fluorescein isothiocyanate-labeled antidigoxigenin antibody, and then analyzed by FACS. Bold lines, binding of the MAA lectin; narrow lines, binding of the SNA lectin; shaded profiles, negative control (no lectin added).

Detail Description Paragraph - DETX (51):

[0066] Generation of **Lectin-Resistant** Cell Lines.

Detail Description Paragraph - DETX (59):

[0074] Generation of **Lectin-Resistant** Cell Lines.

Detail Description Table CWU - DETL (2):

2TABLE 1 Replication of influenza viruses in **lectin-resistant** cell lines*
Titer (TCID₅₀/ml) Cell line AM2AL3 K4 MDCK 1.8 .times. 10^{sup.9} 5.6 .times. 10^{sup.4} MDCK-Sn10 5.6 .times. 10^{sup.8} 3.2 .times. 10^{sup.4} MDCK-Ma 1.8 .times. 10^{sup.8} 5.6 .times. 10^{sup.3} *The susceptibility of each cell line was determined by infecting cells with AM2AL3 or K4 with virus and determining the dose required to infect 50% of tissue culture cells (TCID₅₀).

Claims Text - CLTX (13):

12. A method to isolate a cell that has decreased levels of receptors for influenza virus, comprising: a) contacting a population of cells permissive for influenza virus replication and sensitive to lectin or agglutinin growth inhibition with an amount of lectin or agglutinin so as to yield cells that are **resistant to growth inhibition by the lectin** or agglutinin, wherein the lectin or agglutinin specifically binds sialic acid; and b) isolating a **lectin- or agglutinin-resistant** cell having decreased levels of receptors for influenza virus.

Claims Text - CLTX (28):

27. A method of using a host cell having decreased levels of sialic acid containing host cell receptors for influenza virus, comprising: a) contacting the isolated mutant cell of claim 1 or the resistant cell of claim 22 with an amount of an influenza virus having substantially wild-type levels of sialidase activity so as to yield progeny virus; and b) serially propagating the progeny virus with the mutant cell of claim 1 or the resistant cell of claim 22 so as to yield adapted viruses which efficiently replicate in the mutant cell, the

lectin-resistant cell or the agglutinin-resistant cell.

US-PAT-NO: 6251670

DOCUMENT-IDENTIFIER: US 6251670 B1

TITLE: Method of culturing cells in suspension using lectins

DATE-ISSUED: June 26, 2001

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|---------------------|----------|-------|----------|---------|
| Yoshimoto; Tanihiro | Kanazawa | N/A | N/A | JP |
| Takamatsu; Hiroyuki | Kanazawa | N/A | N/A | JP |

APPL-NO: 09/ 340035

DATE FILED: June 28, 1999

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | APPL-DATE |
|---------|-----------|---------------|
| JP | 10-182450 | June 29, 1998 |

US-CL-CURRENT: 435/383, 435/325 , 435/346 , 435/404

ABSTRACT:

An object of the present invention is to provide a method of enabling perfusion culture efficiently and simply by agglutinating cells with Lectin, which is a naturally-occurring agglutinin, thereby separating the cells and the culture medium. According to the method of the present invention, lectin is added to a culture medium to quickly agglutinate and precipitate the cells, thereby separating the culture medium and the cells. Hence, it is easy to remove old culture medium and replenish with fresh culture medium. Accordingly, if the method of the present invention is used, the perfusion culture is performed automatically and on an industrial scale.

8 Claims, 6 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 4

----- KWIC -----

US Patent No. - PN (1):
6251670

Other Reference Publication - OREF (7):

C. Campbell et al, (Abstract) "A Dominant Mutation to **Ricin Resistance** in Chinese Hamster Ovary Cells Induces UDP-GLcNAc: Glycopeptide Beta-4-N-Acetylglucosaminyltransferase III Activity" Journal: J. Biol. Chem., vol. 259, Issue: 21, pp. 13370-13378 (1984).

Other Reference Publication - OREF (28):

C. Jones, (Abstract) "Increased Cytotoxicity of Normal Rabbit Serum for **Lectin-Resistant** Mutants of Animal Cells", Journal: J. Exp. Med., vol. 160, Issue: 4, pp. 1241-1246 (1984).

Other Reference Publication - OREF (58):

A.G. Tonevitsky et al (Abstract) "Hybridoma Cells Producing Antibodies Against A-Chain of Mistletoe **Lectin I Are Resistant** to This Toxin", Journal: Immunol. Lett., vol. 46, Issue: 1-2, pp. 5-8 (1995).

US-PAT-NO: 6224858

DOCUMENT-IDENTIFIER: US 6224858 B1

TITLE: Hepatocytes transduced with a retroviral vector
comprising splice sites and methods of expression

DATE-ISSUED: May 1, 2001

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|----------------------|-----------|-------|----------|---------|
| Mulligan; Richard C. | Lincoln | MA | N/A | N/A |
| Wilson; James M. | Ann Arbor | MI | N/A | N/A |

APPL-NO: 08/ 488424

DATE FILED: June 7, 1995

PARENT-CASE:

This is a divisional application of application Ser. No. 07/938,260, filed Oct. 22, 1992, now U.S. Pat. No. 5,521,076, which is a CIP of application Ser. No. 07/152,749, filed Feb. 5, 1988, now abandoned.

RELATED APPLICATION

This is a continuation-in-part of U.S. Ser. No. 07/152,749, filed February 5, 1988.

US-CL-CURRENT: 424/93.21, 424/93.2 , 435/320.1 , 435/325 , 435/370

ABSTRACT:

Genetically engineered or transduced hepatocytes which express genetic material of interest introduced or incorporated into them, as well as methods of producing, transplanting and using the genetically engineered hepatocytes. The genetic material of interest can be incorporated through use of a vector, such as a recombinant retrovirus, which contains the genetic material of interest, or by other means.

18 Claims, 39 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 15

----- KWIC -----

US Patent No. - PN (1):
6224858

Detailed Description Text - DETX (31):

Hepatocyte-specific transduction may be possible if modified virions that are specifically internalized by the ASGP receptor are developed and used. For example, it is possible to use viruses whose envelope protein is devoid of sialic acid, thereby rendering them ligands for the ASGP receptor. One approach is to enzymatically remove the terminal sialic acids from intact virions with neuraminidase. Alternatively, it is possible to construct viral producer lines with genetically modified envelope genes that code for glycoproteins with terminal galactose residues. It is possible to construct chimeric envelope genes that encode fusion proteins in which the carboxy terminal sequences are derived from the 3' portion of the envelope gene and the amino terminal sequences are derived from genes of known ligands for ASGP receptor. In addition, it is possible to use lectin-resistance selection systems to isolate mutants of the viral producer lines that are incapable of adding terminal sialic acids to N-linked chains. Viruses produced from these lines should bind to the ASGP receptor. Transduction of cells that express ASGP receptor with these modified virions can be tested in vitro and in vivo.

US-PAT-NO: 5494790

DOCUMENT-IDENTIFIER: US 5494790 A

See image for Certificate of Correction

TITLE: .alpha.-3 sialyltransferase

DATE-ISSUED: February 27, 1996

INVENTOR-INFORMATION:

| NAME | CITY | STATE | ZIP CODE | COUNTRY |
|--------------------|------------|-------|----------|---------|
| Sasaki; Katsutoshi | Machida | N/A | N/A | JP |
| Watanabe; Etsuyo | Kawasaki | N/A | N/A | JP |
| Nishi; Tatsunari | Machide | N/A | N/A | JP |
| Sekine; Susumu | Sagimihara | N/A | N/A | JP |
| Hanai; Nobuo | Sagimihara | N/A | N/A | JP |
| Hasegawa; Mamoru | Kawasaki | N/A | N/A | JP |

APPL-NO: 08/ 309985

DATE FILED: September 20, 1994

PARENT-CASE:

This application is a division of application Ser. No. 07/991,587, filed Dec. 16, 1992, now U.S. Pat. No. 5,384,249.

FOREIGN-APPL-PRIORITY-DATA:

| COUNTRY | APPL-NO | APPL-DATE |
|---------|----------|-------------------|
| JP | 3-333661 | December 17, 1991 |
| JP | 4-091044 | April 10, 1992 |

US-CL-CURRENT: 435/6, 435/193 , 435/252.33 , 435/320.1 , 435/85 , 536/23.2

ABSTRACT:

There are provided a novel .alpha.2.fwdarw.3 sialyltransferase expressed by a cloned gene from animal cells, a cDNA encoding the .alpha.2.fwdarw.3 sialyltransferase, a method for detecting or suppressing the expression of an .alpha.2.fwdarw.3 sialyltransferase by use of said cDNA, a recombinant vector containing said cDNA, a cell containing said vector, and their production processes.

22 Claims, 36 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 29

----- KWIC -----

US Patent No. - PN (1):
5494790

Brief Summary Text - BSTX (21):

There is no case where glycosyltransferase can be cloned using **lectin-resistance** as an index. From the studies on various **lectin-resistant** mutants of CHO cell, it has been revealed that there are cases where new glycosyltransferase is expressed, where the activity of a certain glycosyltransferase disappears, and where synthesis of sugar nucleotide or its transfer to Golgi body is inhibited [Pamela Stanley et al.: Methods in Enzymology, 96, 157]. Therefore, it is considered that cloning of glycosyltransferase can be performed using **lectin-resistance** as index by introducing a gene derived from a cell expressing glycosyltransferase to be cloned into CHO cell or **lectin-resistant** mutants of CHO cell [Ravindra Kumar et al.: Mol. Cell. Biol., 9, 5713 (1989)]. James Ripka et al. have tried to clone N-acetylglucosaminyltransferase I by introducing human genomic DNA derived from A431 cell into **lectin-resistant** mutants of CHO cell (LecI) using **resistance to lectin** concanavalin A as an index. However, they could not clone glycosyltransferase by the screening method using **lectin-resistance** as an index [James Ripka et al.: Biochem. Biophys. Res. Commun., 159, 554 (1989)]. Heffernan et al. have cloned mouse sialic acid hydroxylase using **resistance to lectin** WGA (wheat germ agglutinin) as an index by introducing cDNA library into CHO cell [Michael Heffernan et al.: Nucleic Acids Res., 19, 85 (1991)] which was made to produce large T antigen of polyoma [Michael Heffernan et al.: Glycoconjugate J., 8, 154 (1991)]. However, there is no report in which glycosyltransferase could be cloned in a screening system using the **lectin-resistance** as an index. In addition, with respect to hosts, Stanley, Ripka, Heffernan et al. all used CHO cell or **lectin-resistant** mutants of CHO cell as a host.

Detailed Description Text - DETX (28):

As an animal cell used in the above method, any animal cell can be used so long as cDNA encoding .alpha.2.fwdarw.3 sialyltransferase of the present invention can be expressed in the animal cell. For example, human histiocytic leukemia cell line TYH [Haranaka et al.: Int. J. Cancer, 36, 313 (1985)], human melanoma cell line WM266-4 (ATCC CRL1676) and the like are used. As a vector in which cDNA synthesized using mRNA extracted from these cells as a template is incorporated, any vectors can be used in which said cDNA can be incorporated and expressed. For example, pAMoERC3Sc and the like are used. As an animal or insect cell in which cDNA library constructed using said vector is introduced, any cells can be used in which said cDNA library can be introduced and expressed. For example, human Namalwa cell [Hosoi et al.: Cytotechnology, 1, 151 (1988)] and the like can be used. As lectin to be used in the present invention, any lectins can be used which can inhibit the growth of cell in which cDNA is introduced. For example, Ricinus communis 120 lectin and the like are used. After **resistance of host cell to lectin** is determined, the lectin is used in such the concentration as can inhibit the growth of host

cell. Plasmid having cDNA encoding .alpha.2.fwdarw.3 sialyltransferase of the present invention or DNA fragment containing the cDNA part is recovered from cells which grow in the presence of lectin by the known method, for example, Hirt method [Robert F. Margolskee et al.: Mol. Cell. Biol., 8, 2837 (1988)]. As a plasmid having cDNA encoding the enzyme of the present invention, there are, for example, pUC19-LEC, pUC19-WM17 and the like. Escherichia coli HB101/pUC19-LEC containing pUC19-LEC and Escherichia coli HB101/pUC19-WM17 have been deposited with Fermentation Research Institute, Agency of Industrial Science and Technology, 1-3, Higashi 1 chome Tsukuba-shi Ibaraki-ken 305, Japan on Oct. 29, 1991 and on Sep. 22, 1992 under the Budapest Treaty, and have been assigned the accession number FERM BP-3625 and FERM BP-4013, respectively.

Detailed Description Text - DETX (30):

DNA encoding .alpha.2.fwdarw.3 sialyltransferase of the present invention obtained in the above process is inserted downstream of appropriate promoter to construct recombinant vector which is introduced in a host cell, and the resulting cell is cultured to obtain .alpha.2.fwdarw.3 sialyltransferase of the present invention. As a host cell, any host cells can be used to which recombinant DNA techniques have been applied such as procaryotic cells, animal cells, yeasts, fungi, insect cells and the like. For example, there are Escherichia coli cell as procaryotic cell, CHO cell which is Chinese hamster ovary cell, COS cell which is monkey cell, Namalwa cell which is human cell and the like as animal cell. In particular, direct expression system using Namalwa cell as a host cell is suitably used due to such the advantages that efficiency which introduces cDNA library in Namalwa cell as a host cell is extremely high, the introduced plasmid (cDNA library) can exist extrachromosomally, and plasmid is easily recovered from the resultant lectin resistant strain.

Detailed Description Text - DETX (141):

Namalwa cells conditioned for serum-free media (KJM-1 strain) (Hosoi et al., Cytotechnology, 1, 151 (1988)) were cultured in the presence of Ricinus communis 120 lectin at various concentrations, and the resistance of the KJM-1 strain to Ricinus communis 120 lectin was examined. The KJM-1 strain was suspended in RPMI1640.ITPSGF medium (RPMI1640 medium (Nissui Seiyaku) containing a 1/40 volume of 7.5% NaHCO.sub.3, 200 mM L-glutamine solution (GIBCO) of 3% in volume, penicillin-streptomycin solution (GIBCO; 5000 units/ml penicillin and 5000 .mu.g/ml streptomycin) of 0.5% in volume, 10 mM HEPES, 3 .mu.g/ml insulin, 5 .mu.g/ml transferrin, 5 mM sodium pyruvate, 125 nM sodium selenate, 1 mg/ml galactose, and 0.1% (w/v) prulonic F68) to give a concentration of 5.times.10.sup.4 cells/ml, and the suspension was distributed in 200-.mu.l portions into wells of a 96-well microtiter plate. Various concentrations of Ricinus communis 120 lectin (Seikagaku Kogyo) were added thereto in 1/100 volumes, and the plate was incubated in a CO.sub.2 incubator at 37.degree. C. for 1 to 2 weeks. As the result, it was found that the minimum concentration of Ricinus communis 120 lectin to cause complete inhibition of the KJM-1 strain growth was 50 ng/ml. Four million cells of the KJM-1 strain were examined, and the natural occurrence of Ricinus communis 120 lectin resistant strain was not observed.

Detailed Description Text - DETX (153):

The above plasmid was introduced into the KJM-1 strain by electroporation (Miyaji et al., Cytotechnology, 3, 133 (1990)) at a proportion of 4 .mu.g per 1.6.times.10.sup.6 cells. After the introduction of plasmid, these cells were suspended in 8 ml of RPMI1640.ITPSGF medium, and the cells were incubated in a CO.sub.2 incubator at 37.degree. C. for 24 hours. Then, the cells were supplemented with G418 (GIBCO) to give a concentration of 0.5 mg/ml and further cultured for 5 to 7 days to give transformants. The obtained transformants were suspended in RPMI1640.ITPSGF medium containing Ricinus communis 120 lectin (50 ng/ml) to give a concentration of 5.times.10.sup.4 cells/ml, and the cells were distributed in 200-.mu.l portions into wells of a 96-well microtiter plate. The cells were cultured in a CO.sub.2 incubator at 37.degree. C. for 2 to 3 weeks, and 7 strains were obtained which had become resistant to a Ricinus communis 120 lectin. From this resistant strain, a plasmid was isolated according to the Hirt method (Robert F. Margolskee et al., Mol. Cell. Biol., 8, 2837 (1988)), and Escherichia coli strain LE392 was transformed with this plasmid by electroporation (William J. Dower et al., Nucleic Acids Res., 16, 6127 (1988)). From this transformant, a plasmid was prepared using ϕ-plasmidϕ-maxi kit (trade No. 41031; Qiagen), and its structure was examined by restriction enzyme digestion to find that it contained about 1.9 kb cDNA. The cDNA containing plasmid was designated as pAMoERL. When this plasmid was also introduced into the KJM-1 strain by the above method, this strain became resistant to Ricinus communis 120 lectin; it was therefore found that this cDNA is a gene responsible for lectin resistance. The KJM-1 strain containing the plasmid pAMoERL was able to grow even in the presence of 200 ng/ml of Ricinus communis 120 lectin.

Detailed Description Text - DETX (163):

The nucleotide sequence of the deletion plasmid obtained above was determined using the Taq DyeDeoxy terminator cycle sequencing kit (trade No. 401113; Applied Biosystems). The determined nucleotide sequence is shown in the Sequence Listing (Seq. ID: 1). Also, it was found from the corresponding-amino acid sequence that this protein has a common structure to glycosyltransferase (hereinafter abbreviated to GT). That is, this protein seems to have a structure where 8 amino acids in the N-terminal portion are put out at the cytoplasm side, a highly hydrophobic region consisting of the subsequent 18 amino acids is used for binding to the membrane, and most of the remaining C-terminal portion including -the catalytic site is exposed to the internal cavity of the Golgi's apparatus. The comparison of amino acid sequence between this protein and other GTs made clear that this protein has a certain homology with rat .alpha.2.fwdarw.6 sialyltransferase. For these reasons, it is considered that Ricinus communis 120 lectin resistance gene encodes GT.

Detailed Description Text - DETX (164):

5. Measurement of .alpha.2.fwdarw.3 sialyltransferase activity of the KJM-1 strain having expression plasmid for Ricinus communis 120 lectin resistance gene

Detailed Description Text - DETX (170):

The comparison of HPLC pattern between the KJM-1 strain having the plasmid

pAMoERL and the KJM-1 strain having the plasmid pAMoERC3Sc made clear that both the strains gave approximately the same peak 1 but the strain having the pAMoERL strain gave a significantly higher peak 2 than that of the strain having the pMoERC3Sc. The ratio of peak 2 to peak 1 for the KJM-1 strain having the pAMoERL was 6 to 7 times greater than that for the KJM-1 strain having the pAMoERC3Sc as the vector (see FIG. 17). From these results, it was shown that this *Ricinus communis* 120 lectin resistance gene is an .alpha.2.fwdarw.3 sialyltransferase gene and that oligosaccharides with sialic acid added can be produced using .alpha.2.fwdarw.3 sialyltransferase encoded in the said gene.

Detailed Description Text - DETX (245):

The above plasmid was introduced into the KJM-1 strain by electroporation (Miyaji et al., Cytotechnology, 3, 133 (1990)) at a proportion of 4 .mu.g per 1.6.times.10.sup.6 cells. After the introduction of plasmid, these cells were suspended in 8 ml of RPMI1640.ITPSGF medium, and the cells were cultured in a CO.sub.2 incubator at 37.degree. C. for 24 hours. Then, the cells were supplemented with G418 (GIBCO) to give a concentration of 0.5 mg/ml and further cultured for 5 to 7 days to obtain transformants. The obtained transformant was suspended in RPMI1640.ITPSGF medium containing *Ricinus communis* 120 lectin (50 ng/ml) to give a concentration of 5.times.10.sup.4 cells/ml, and the suspension was distributed in 200-.mu.l portions into wells of a 96-well microtiter plate. The cells were cultured in a CO.sub.2 incubator at 37.degree. C. for 4 weeks, and a certain strain was obtained which had become resistant to *Ricinus communis* 120 lectin. After culturing of this resistant cell, a plasmid was isolated from about 5.times.10.sup.6 cells according to the Hirt method (Robert F. Margolskee et al., Mol. Cell. Biol., 8, 2837 (1988)). The isolated plasmid was introduced into *Escherichia coli* strain LE392 by electroporation (William J. Dower et al., Nucleic Acids Res., 16, 6127 (1988)) to give an ampicillin resistant transformant. From this transformant, a plasmid was prepared using >plasmid<maxi kit (Qiagen), and its structure was examined by restriction enzyme digestion to find that it contained about 1.9 kb cDNA. The cDNA containing plasmid was designated as pAMoPRWM17. When this plasmid was also introduced into the KJM-1 strain by the above method, the transformant became resistant to *Ricinus communis* 120 lectin; it was therefore found that this cDNA is a gene responsible for lectin resistance. The KJM-1 strain containing the plasmid DAMoPRWM17 was able to grow even in the presence of 200 ng/ml *Ricinus communis* 220 lectin.